PBDE, 2,2',4,4',5-PENTABROMODIPHENYL ETHER, CAUSES PERMANENT NEUROTOXIC EFFECTS DURING A DEFINED PERIOD OF NEONATAL BRAIN DEVELOPMENT

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame-retardant additives in polymers, especially in the manufacture of a great variety of electrical appliances, including television and computer casing, building materials, and textiles (1). One of the earliest reports of PBDE in our environment came in 1981 (2). PBDEs are persistent compounds that appear to have an environmental dispersion similar to that of PCB and DDT. PBDEs have been found in various wildlife species, human adipose tissue and recently also in human plasma samples (3,4,5). The PBDEs are now also seen to increase in mother's milk and one of the dominating PBDE congener is 2,2',4,4',5-pentabromodiphenyl ether (PBDE 99) (6,7).

In mammals, the fetus can be directly exposed during gestation via maternal intake of toxic agents. During the neonatal period, offspring may be affected by toxic agents by ingesting mother's milk, or be directly exposed to xenobiotics. In many mammalian species a rapid growth of the brain occurs during perinatal development, the so-called "brain growth spurt" (8). In the human, this period begins during the third trimester of pregnancy and continues throughout the first 2 years of life. In mouse and rat this period is neonatal, spanning the first 3-4 weeks of life, during which the brain undergoes several fundamental phases that transform the feto-neonatal brain into that of the mature adult (8,9).

In a recent study we have seen that neonatal exposure to PBDE 99 and PBDE 47 can cause permanent aberrations in spontaneous behaviour that seems to worsen with age (10). Furthermore, neonatal exposure to PBDE 99 also affected learning and memory functions in adult animals. In several reports we have shown that low-dose exposure of environmental toxic agents such as PCB, DDT, pyrethroids, organophosphates, paraquat and nicotine, during "BGS", in neonatal mice can lead to disruption of the adult brain function, and also to an increased susceptibility to toxic agents at adult ages (11). The studies have also shown that there is a critical phase in the neonatal development, when the maturational processes of the developing brain and CNS are at a stage of critical vulnerability, that these persistent effects are induced.

In view of an increasing amount of PBDEs in mother's milk and in the environment, the present study was undertaken to investigate whether there is a critical and limited phase in the neonatal development of the brain for induction of persistent neurotoxic effects of PBDE 99. Furthermore,

uptake and retention studies of ^{14}C-labelled PBDE 99 in the neonatal mouse brain were performed to measure the amount of PBDE in the brain at different neonatal ages.

Materials and Methods

Unlabelled and ^{14}C-labell 2,2',4,4',5-pentabromodiphenyl ether (PBDE 99) were synthesized at the Wallenberg Laboratory (12,13,14), University of Stockholm, Sweden.

The substances were orally administered to neonatal NMRI-mice as one single oral dose at an age of either 3-, 10-, or 19-days. In the behavioural study mice received 8 mg (14 µmol) 2,2',4,4',5-pentabromodiphenylether (PBDE 99)/kg body weight, and in the uptake and retention study mice received 1.5 MBq [^{14}C]2,2',4,4',5-pentabromodiphenyl/kg body weight. Mice serving as controls received 10 ml/kg body weight of the 20% fat emulsion vehicle in the same manner.

Spontaneous behaviour: the test was performed in male mice at the age of 4 months. The test measures locomotion: horizontal movement, rearing: vertical movement, and total activity: all types of vibration within the test cage, i.e. those caused by mouse movements, shaking (tremors) and grooming.

Uptake and retention: the amounts of radioactivity found in the brain 24 h and 7 days after administration of ^{14}C-labelled PBDE 99 at the three different ages were analysed.

Results and Discussion

A significant behavioural aberration was observed in adult mice given PBDE 99 (8.0 mg/kg body weight) at an age of either 3 or 10 days, but in mice receiving PBDE 99 on postnatal day 19 no significant change was seen. The adult mice (4 months) exposed on postnatal day 10 displayed a non-habituating behavioural profile, as earlier seen for this congener (10) but also for some PCB congeners (15,16,17), namely a hypoactive condition during the first part of the 60-min test period, while toward the end of the period they became demonstrably hyperactive. This change was also seen in mice exposed on postnatal day 3, but to a lesser degree.

The amount of a toxic agent that is present in the brain at different neonatal ages might vary. Previous studies have shown a pronounced retention of some lipophilic chlorinated hydrocarbons or their metabolites,[such as DDT, PCB 52 (2,2',5,5'-tetrachlorobiphenyl) and PCB 153 (2,2',4,4'5,5'-hexachlorobiphenyl)] in the brain when administered on postnatal day 10 (see 17). The retention of PBDE 99 was found to be similar to that observed after neonatal exposure to PCB 52, PCB 153 and DDT. Furthermore, the observed retention of PBDE 99 in mice exposed on day 3 indicates that the behavioural disturbances seen in these animals at adult age might be attributable to the amount of PBDE 99 present on day 10 being enough to induce behavioural disturbances.

The observed critical window for the induction of irreversible effects of PBDE 99 is similar to that in our earlier studies where permanent changes in spontaneous behaviour and in cholinergic receptors has been observed following neonatal exposure to DDT, diisopropylfluorophosphate (DFP, organophosphate), nicotine and PCB (11,17,18,19).

In earlier studies we have seen that neonatal exposure to some PCBs affects the cholinergic system, e.g. changes in cholinergic receptors and altered behaviour response to nicotine (see 17). The

cholinergic system plays an important role in many behavioural phenomena, e.g. learning and memory, neurological syndromes, audition, vision, and aggression (see 20). Pharmacological manipulations of the cholinergic system have been correlated in several studies with altered cognitive behaviour. In order to explore whether the observed changes in spontaneous behaviour in adult mice neonatally exposed to PBDE 99 (8 mg/kg body weight) would include effects on the cholinergic system, the behavioural response of adult animals to a cholinergic (nicotine) agent was also studied. In mice treated neonatally on postnatal day 10 with PBDE 99 showed the same non-habituating behaviour at the age of 4 months as earlier described (10), but the response to a low dose of nicotine was quite the reverse, compared with the controls. In control animals, hyperactivity was seen after 80 µg nicotine base, whereas the PBDE 99-treated mice were obviously hypoactive. This response to nicotine in the PBDE 99-treated mice is the same as we found earlier in mice treated neonatally with PCB 52 (16) and nicotine (21).

In this study we report that the ability of a single oral dose of PBDE 99 to induce persistent effects on behaviour is limited to a vulnerable period during the "brain growth spurt" ("BGS"). The neurotoxic effects seem also to involve changes in the cholinergic system. When considering the critical window for induction of permanent neurotoxic derangement during the "BGS" in mice, a corresponding period in humans starts during the third trimester of gestation and continues for several months after birth. Taken together with the increasing concentration of PBDEs in mother's milk, call for future research of PBDEs as potential neurotoxicants.

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